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iGEM TU/e 2016
Biomedical Engineering

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Gibson Assembly

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Gibson Assembly

1 NEBuilder HiFi DNA Assembly Reaction

Estimated bench time: 30 minutes
Estimated total time: 50 minutes
Purpose: The assembly of multiple inserts within the vector of choice.

1.1 Materials

- Autoclaved dH_2O
- Bucket with ice
- gBlocks
- Linearized vector
- NEBuilder HiFi DNA Cloning Kit
- PCR machine

1.2 Setup & Protocol

- Look up the following parameters to complete the table. The molecular weight of the double stranded vector and gBlocks can be determined using a DNA molecular weight calculator online.

Component	**Molecular weight (Da)**	**Concentration (ng/µl)**
Linearized vector		
gBlock 1		10
Additional gBlocks		10

- The amount of pmol for the Gibson assembly has to be between the following ranges:
  1 or 2 gBlocks: 0.02 – 0.5 pmol (with a vector : gBlock ratio of 1:2 or 1:3)
  4 to 6 gBlocks: 0.02 – 1.0 pmol (with a vector : gBlock ratio of 1:2 or 1:3)

  pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)

- Set up the following reaction on ice:

Component	**Value in mixture**	**Volume (µl)**
Linearized vector	50-100 ng	
gBlock 1 (10 ng/µl)	3-fold excess (3x pmol vector)	
Additional gBlocks (10 ng/µl)	3-fold excess	
NEBuilder HiFi DNA Assembly Master Mix	10 µl	10
Autoclaved dH_2O	Till 20 µl	
Total volume		20

The volume of linearized vector and gBlock can be calculated with:

$$Volume\ (\mu l) = \frac{amount\ (pmol) * molecular\ weight\ (Da)}{concentration\ (\frac{g}{l})}$$

- For 2-3 gBlocks: Incubate the samples at 50 °C for 15 minutes using the PCR machine.
- For 4-6 gBlocks: Incubate the samples at 50 °C for 15 minutes using the PCR machine.
- After incubation, store the samples on ice for subsequent transformation.

2 Transformation into NEB 5-alpha competent E. Coli

Estimated bench time: 1 hour
Estimated total time: 2 hours
Purpose: Transformation of the assembled vector into competent cells. These cells can be used for subsequent plasmid amplification.

During transformation with NEB 5-alpha you are working with bacterial cells. Therefore, you need to work near the Bunsen burner flame, prohibiting the use of gloves during this step.

2.1 Materials

- Bucket with ice
- Brunsen Burner
- Heat/shaking-block
- Incubator
- LB-agar plates supplemented with the correct antibiotic
- NEB 5-alpha competent cells
- Pipettes and tips
- Plasmids to be transformed
- SOC solution
- Water bath

2.2 Setup & Protocol

- Thaw the NEB 5-alpha competent E. Coli on ice such that all ice crystals disappear.
- Add 2 µl of the chilled samples to the competent cells. Mix gently by stirring with your pipette. Do not vortex nor pipette up and down.
- Place mixture on ice for 30 minutes. Do not mix.
- Heat shock the cells for exactly 30 seconds at 42 °C.
- Transfer the tubes directly to ice for 2 minutes.
- Add 950 µl of room-temperature SOC media to the tube.
- Incubate for 60 minutes at 37 °C and 300 rpm.
- Spread 100 µl of the cells onto the agar plates while working near the Bunsen burner flame. Incubate overnight at 37 °C.

3 References & Acknowledgements

This protocol was adapted from the NEBuilder HiFi Assembly Cloning Kit's manual with help from 2015 team of iGEM TU Eindhoven.